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Cell-Based Therapies for Diabetic Retinopathy

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Abstract

Autologous endothelial progenitor cell (EPC) populations represent a novel treatment for therapeutic revascularization and vascular repair for diabetic patients with complications including diabetic retinopathy. Current therapies are applicable to late-stage disease and carry significant side effects, whereas cell-based therapy may provide an alternative by repairing areas of vasodegeneration and reversing ischemia. However, EPCs from diabetic patients with vascular complications are dysfunctional. Moreover, the diabetic environment poses its own challenges and complicates the use of autologous EPCs. Before EPCs become the ideal “cell therapy,” the optimal EPC must be determined, any functional dysfunction must be corrected prior to use, and the diabetic milieu will require modification to accept the EPCs. This review describes the rationale for harnessing the vascular reparative properties of EPCs with emphasis on the molecular and phenotypic nature of healthy EPCs, how diabetes alters them, and novel strategies to improve dysfunctional EPCs.

Keywords

Diabetes; Diabetic retinopathy; Endothelial progenitor cell; Stem cell therapy; Circulating angiogenic cells; Circulating endothelial cells; Outgrowth endothelial cell; Embryonic stem cells; Renin-angiotensin-aldosterone system

Introduction

Ischemia is characterized by a restriction in blood flow to tissues and organs, depriving them of vital oxygen and nutrients and removal of metabolites, and represents a hallmark of diabetic vascular complications. Treatment of micro- and macrovascular complications, the leading causes of morbidity and mortality in diabetic patients, requires correction of tissue ischemia; however, typical revascularization strategies, such as bypass procedures and stenting, fail in these patients because diabetic patients suffer from “small vessel” disease. Although diabetic complications may be triggered by endothelial dysfunction, the lack of endothelial regeneration/repair contributes to the progression of these complications.

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Endothelial progenitor cells (EPCs) are the key cells responsible for the maintenance and repair of the vasculature. Diabetes-related EPC dysfunction is now appreciated as closely linked to the impaired healing response experienced by many diabetic patients and the loss of function of these cells is clearly contributory to vasodegenerative changes observed in diabetic macro- and microvasculature [1–3]. Although EPC therapy represents a potentially valuable option for diabetic patients with complications, this “therapeutic” population of cells is often dysfunctional. Specifically, circulating EPC numbers are reduced in diabetic patients with complications, and EPCs show impaired function in both in vitro and in vivo assays of angiogenesis [3–6]. Therefore, the use of autologous cells is less feasible unless the cells are first modified to correct their intrinsic dysfunction.

The Origin of EPCs Remains a Controversy

EPCs have been long described as bone marrow–derived cells. Although most endothelium probably arises from division of neighboring cells, use of chimeric transplant mouse models has shown that approximately 1% of the endothelium is contributed directly by the bone marrow [7]. Whereas CD34⁺ and circulating angiogenic cell (CAC) subgroups have been proven to be marrow derived [8–10], the origin of the outgrowth endothelial cell (OEC) subgroup remains somewhat less certain, and the precise profile is yet to be agreed upon. The first report confronting this issue suggested that although circulating endothelial cells are derived from the vessel wall, OECs arise from bone marrow–derived circulating angioblasts [11]. Later, the existence of a “vasculogenic zone” in the wall of adult human blood vessels, either in bone marrow or other tissues, that contains OECs has been suggested [12]. Therefore, now when the term endothelial progenitors is used, the origin of these cells could be the endothelium itself [13] or the bone marrow. Some investigators purport that within vessel wall–derived cultures [14], the complete hierarchy of endothelial progenitor cells can be identified. Other studies indicate that OECs are not progenitors at all but are rather culture artifacts arising from in vitro conditions [15], albeit with properties that could be exploited for therapeutic use. Endothelial progenitors have been isolated from different tissues such as liver, muscle, and dermis, suggesting the existence of diverse vascular stem cell niches, reviewed by Watt et al. [16].

What Role Does the EPC Play in Vascular Repair?

Although there is still an argument about the true origin of the EPC, most stem cell researchers agree about how the EPC repairs. All therapeutic strategies that use EPCs take advantage of their ability to deliver cytokines and growth factors to diseased tissue to foster revascularization and correction of tissue ischemia. Cell-based tissue regeneration requires effective revascularization [17, 18]. For successful revascularization to occur, the therapeutic cells must mobilize from their niche in the bone marrow and enter the circulation in response to hypoxic stimuli released by ischemic tissue; healthy EPCs home specifically to areas of injury, and participate in vessel formation/repair with the resident vasculature [19]. In diabetes this response to hypoxia is altered and EPCs have reduced ability to mobilize from their resident niche into the circulating blood [20••]. The mechanisms involved in mobilization include stimulatory factors such as erythropoietin (EPO) [21], vascular endothelial growth factor [22], and granulocyte colony–stimulating factor (G-CSF) [23]. Signals released from apoptotic endothelial cells are also important for EPC recruitment to sites of vascular damage [24].

Another key hypoxia-regulated factor is stromal cell–derived factor-1 (SDF-1) and its receptor CXCR4, which is known to be essential for EPC migration to areas of ischemic damage [25]. Our group showed that blocking the CXCR4/SDF-1 signaling pathway inhibits EPC homing and results in their attenuated participation in retinal neovascularization [26].

What is the Best EPC Population for Cell Therapy?

As suggested above, there are many possible EPC populations that can be used therapeutically. CACs or early endothelial progenitor cells (eEPCs) [27] and the more recent EPC-type cell described by Hill et al. [28] as colony-forming units appear predominantly hematopoietic in nature with a typical myelomonocytic phenotype. These cells have been shown to promote neovascularization in ischemic tissues [29–31], suggesting that although these particular EPCs may not directly incorporate into vessels, they foster repair by secretion of cytokines and growth factors such as hepatocyte growth factor [32], interleukin-8, G-CSF, and granulocyte-macrophage CSF [33]. This paracrine support nourishes the resident endothelium and other endothelial progenitor populations fostering their survival and even their proliferation. Some investigators suggest that CACs and eEPCs represent the same population and that as “paracrine modulators” they are not able to incorporate directly into vessels to “build” new blood vessels directly.

In contrast to CACs and eEPCs, OECs have been shown to have “direct” involvement in vascular repair by forming well-perfused neovessels when injected subcutaneously within matrigel plugs [34, 35]. OECs also support resident vessels by releasing paracrine factors [36]. OECs are “endothelial” cells because they express CD105, CD146, von Willebrand factor, vascular endothelial growth factor receptor 2, look like endothelial monolayers, form tubes in vitro, and incorporate into mature blood vessels in vivo [37••]. Therefore, OECs may serve as “building blocks” of the vasculature and may be directly involved in forming the endothelial lining of damaged vasculature.

Thus although CACs and OECs are both commonly cited as EPCs and are both involved in vascular repair and tissue regeneration, their mechanisms of action are distinct. Consequently, it has been demonstrated that transplantation of mixed EPCs (CACs+OECs) results in synergistic neovascularization using a murine hindlimb ischemic model [38].

Are More than Endothelial Progenitors Needed?

Much of the work from our laboratory and others supports that the therapeutic vasculogenesis process also involves pericytes, which play a fundamental role in vessel maturation and stability [39, 40]. Mesenchymal stem cells are also bone marrow–derived cells that can form endothelial cells and facilitate vessel repair [41]. Therapeutic stem cells also include multipotent mesenchymal stem cells [42], cardiac stem cells [43], and pluripotent embryonic stem cells (ESCs) [44]. The safety and efficiency of transplanting cells, especially ESCs with their ethical considerations, uncertain differentiation potential, and inherent risk for teratoma formation, raise concerns surrounding their clinical utility [45].

Human EPC Trials

EPC-linked patient-based studies initially tested bone marrow cells (BMCs) as candidates to reverse ischemic heart disease [46]. Although initial results were promising [47, 48], more recent trials using heterogeneous populations of BMCs have generated variable results including failure to show any beneficial outcome from cell therapy [49]. Nevertheless, there is considerable optimism for the clinical use of EPCs for patients with ischemic disorders [50].

Some argue that the variable outcome from EPC-based clinical studies, to date, is linked to use of unfractionated bone marrow–derived cells or heterogeneous populations of EPCs that are not well characterized, yet an equal number of investigators support the use of heterogeneous populations as representing a more physiologic strategy for replacement of

injured endothelium [51]. This raises several key questions including whether use of homogenous cells with well-defined functional phenotypes is better than mixed populations and whether inflammatory cells are also needed for proper repair of vessels. In the diabetic patient this may be even more complicated because inflammatory cells may be contributing to the pathology. Transplanting an “inappropriate EPC” might actually exacerbate the ischemia rather than facilitate the reparative response.

For example, CACs are enriched for genes involved in inflammation and immune responses [37••]. Therefore, if injected into a proinflammatory microenvironment such as in ischemic diabetic tissues, they might serve to exacerbate the pre-existing pathology [52, 53]. OECs have the least potential to differentiate into an inflammatory cell and this may be most desirable in some settings, but not in others. Therefore, the molecular and functional phenotype of the EPCs is key and is likely to be specific for the vascular bed in need of repair. In addition, the health of the individual at the time of cell therapy has a salient role in the success of the outcome of any cell therapy. All of medicine is becoming personalized and clearly cell therapy is no exception.

Diabetic EPC Dysfunction

In diabetes, retinal vasodegeneration occurs as part of nonproliferative diabetic retinopathy (NPDR). The diabetic environment produces increased levels of reactive oxygen species (ROS) and progenitors undergo a change to produce pathologic cytokines such as monocyte chemoattractant protein-1, tumor necrosis factor- α , interleukin-8, and express increased levels of pathologic-inducible nitric oxide (NO) synthase, rather than endothelial nitric oxide synthase (eNOS) (Fig. 1). Diabetic EPCs have a reduced ability to proliferate and glycated hemoglobin A_{1c} levels are inversely correlated with proliferation [2]. Diabetic EPCs have reduced bioavailable NO due to either decreased eNOS activity/expression or increased generation of ROS via upregulated NADPH oxidase (Fig. 1) [54]. NO-dependent signaling events also play a major role in the mobilization of EPCs from the bone marrow and in their homing to areas of ischemia [55, 56].

Our group has shown that the function of EPCs in diabetic patients can partially be restored by increasing eNOS expression, by using NO donors, or by decreasing NADPH oxidase-dependent ROS production [57, 58]. Whether EPCs are more resistant to oxidative stress than mature endothelium [59] is a matter of debate as recent studies indicate that EPCs remain highly susceptible to the diabetic milieu (Fig. 1) [60]. Aging can impair antioxidant defense enzymes [61] and prolonged oxidant exposure reduces reparative function [62]. However, in addition to increased ROS and reduced bioavailable NO, several other molecular mechanisms have been proposed to explain EPC dysfunction in diabetes, including a reduction of cathepsin L activity [63] and an upregulation of thrombospondin-1 [64].

Diabetic Retinopathy and EPCs

With respect to the retina and proliferative diabetic retinopathy (PDR), recent evidence suggests that high levels of bone marrow-derived EPCs contribute to the pathologic neovascularization of ischemic tissues and are a critical risk factor for the development of these complications. Tan et al. [65] evaluated the functional properties of CD34⁺ CD45⁻ endothelial colony-forming cells (ECFCs) in patients with PDR. Although they found higher levels of circulating CD34⁺CD45⁻ cells in PDR patients compared with controls, the ECFCs from these patients were impaired in their ability to migrate toward SDF-1 and human serum, incorporate into and form vascular tubes with human retinal endothelial cells. They suggested that although the ECFCs from patients with PDR are mobilized into the circulation, they are unable to migrate and repair damaged capillary endothelium [65].

Approaches seeking to reverse EPC defects in diabetic patients have included enhancement of the angiogenic stimulus by increasing EPC mobilization and homing with G-CSF [66] and SDF-1, respectively [26, 67], or use of an NO donor to correct SDF-1-mediated migration defects and promote cell deformability [57]. There is also some evidence to suggest that diabetic EPC dysfunction can be improved/normalized by treatment with rosiglitazone [68] or atorvastatin [69]. All these studies offer insight into the nature of the diabetes defect in EPCs, but it is also important to appreciate that the “host” microenvironment of diabetic vessels is comparatively inhospitable to normal EPCs [52]. Thus, a major concern regarding cell therapy in diabetic individuals is the issue of injecting therapeutic cells into an unreceptive environment. Ingram et al. [70], focusing on progenitors isolated from cord blood, showed that the diabetic intrauterine environment reduced ECFC colony formation, self-renewal capacity, and tube formation in matrigel when compared with nondiabetic controls. Moreover, our group has demonstrated that diabetes-linked modifications to the capillary basement membrane through advanced glycation significantly impairs EPC-mediated reparative function, a dysfunction that can be reversed by encouraging cell interactions with the substrate proteins [71].

How Do EPCs Respond Differently in a Healthy Versus Ischemic Retina?

Nearly a decade ago, we found that bone marrow-derived cells contribute significantly to neovascularization in the ischemic retina [15], and other reports have demonstrated the intravitreal delivery of bone marrow-derived stem cells, Lin[−] hematopoietic stem cells (HSCs). We showed that human CD34⁺ cells, derived from HSCs, can repair ischemic retina [72] and the reparative utility of HSCs has been corroborated by other groups [3], [73]. A subpopulation of cells within the HSC Lin[−] fraction selectively home to retinal astrocytes and stably incorporate into sites adjacent to developing retinal vasculature where they exert vasculotrophic and neurotrophic effects and rescue degenerating retinas [73, 74].

Moreover, using four different models of ischemic vascular damage, we showed that healthy but not diabetic CD34⁺ cells attached close to the retinal vasculature [3]. In this study, there was partial incorporation of injected cells ranging from about 2% for diabetic cells, to about 31% for healthy cells. These studies support the therapeutic potential of EPCs for ischemic retinopathies; however, only healthy cells successfully enhanced revascularization of the retina. Importantly, when diabetic progenitor cells, specifically Sca-1⁺ bone marrow-derived EPCs, were transplanted into diabetic mice, they were converted into deleterious cells, proinflammatory and anti-angiogenic cells, which only served to exacerbate limb ischemia [52]. Hyperglycemia alters EPC differentiation, reduces vasoregenerative potential, and pushes cells towards a proinflammatory phenotype [53]. Therefore, it must always be kept in mind that the use of any bone marrow population when placed into a highly pathologic environment may aggravate, rather than improve, retinal function.

Many Different Progenitors of Myeloid Origin Promote Vascular Repair in the Ischemic Retina

To better characterize and identify the active cell population inside the Lin[−] HSCs, Ritter et al. [75] examined CD44⁺ (hyaluronic acid receptor). CD44^{hi} cells promoted retinal vascular repair in a similar manner to Lin[−] HSC cells, although interestingly, CD44^{hi} cells were further characterized as myeloid progenitors, and on intravitreal injection in the retina of mouse pups undergoing the oxygen-induced retinopathy (OIR) model, these cells differentiated into microglia [75]. Their studies convincingly showed that these myeloid progenitors (CD44^{hi} cells) significantly enhance revascularization of ischemic pup retinas and that this beneficial effect was dependent on hypoxia-inducible factor-1 α expression and upregulation of proangiogenic transcriptional activity in myeloid cells.

Medina et al. [37••] evaluated another myeloid cell population, CACs in the same mouse model, and found that CACs are capable of promoting vascular repair in a paracrine manner by secreting various cytokines. In this same model, our group showed similar results with CD34⁺ cells [3, 76].

Tumor-associated macrophages typically show angiogenic and anti-inflammatory properties [77]. Because intraretinal neovessels are frequently surrounded by macrophages, macrophages—specifically proangiogenic type 2 macrophages—may foster retinal vascular repair. However, in diabetes macrophages may exacerbate the ischemia [68, 78].

Nonmyeloid Cells Contribute to Revascularization of the Ischemic Retina

OECs are fully committed to the endothelial lineage and Medina et al. [37••] tested their potential for reversing ischemic retinopathy. In vitro, OECs closely interact with retinal microvascular endothelial cells through adherens and tight junctions, and they also integrate into retinal vascular networks. Using the OIR model, they demonstrated that OECs directly incorporated into the resident vasculature, significantly decreasing avascular areas and preventing pathologic preretinal neovascularization, concluding that OECs have potential as therapeutic cells to revascularize the ischemic retina [37••].

Options for Mixed Cell Transplants

As mentioned above, different EPC types demonstrate distinct functions regarding revascularization of ischemic tissues so it is not surprising that a “mixed” transplantation of CACs and OECs resulted in superior neovascularization than single cell-type transplantation [38], suggesting a beneficial cross-talk between EPC subtypes through paracrine networks of cytokines and matrix metalloproteinases. Taking this one step further, it may be ideal to use progenitor populations that are destined to replace different cells of a vessel and not only the endothelium. Thus, using pericyte progenitors with endothelial progenitors may promote vessel maturity and stability to a greater degree [79–81]. The retinal vasculature carries the highest pericyte coverage compared with all other vascular beds in the body and the fact that pericyte loss is one the earliest indications of developing diabetic retinopathy argues for the combined use of mural cell precursors and EPCs [82]. Thus, although using a cell such as an OEC that essentially is a transplantable endothelial cell may be good for building endothelium, a more immature progenitor that can generate both endothelial cells and pericytes may be a better alternative for building a blood vessel. A mixed transplantation of EPCs and mesenchymal progenitors has been shown to offer a highly beneficial synergistic effect on neovascularization and vessel stabilization in nonretinal systems [41, 83].

What is Limiting Use of Cell Therapy in Treatment of Diabetic Retinopathy?

Cell-based therapy may represent an exciting alternative strategy to the current end-stage approaches to diabetic retinopathy and other ischemic retinopathies. This approach is designed to target early/intermediate stages of vasodegeneration to enhance vascular repair, reverse ischemia, reduce hypoxic/inflammatory stimuli, and prevent progression to the late, sight-threatening stages of these diseases. This strategy holds promise if a patient’s autologous progenitors could be modified to function properly. This review has described the controversy regarding which cell is ideal for use as cell therapy and has described success with both freshly isolated populations (CD34⁺ cells) or ex vivo expanded populations (eEPCs and OECs) and with well-characterized, homogenous cells as well as mixed populations. But “repairing” the reparative cell in diabetic patients likely will represent the largest hurdle to overcome. Numerous strategies have been already discovered [53, 84, 85], antioxidants [59, 62], peroxisome proliferator-activated receptor- δ agonists,

NO donors [57], EPO [21, 86], and modulation of transforming growth factor (TGF)- β [87••].

Using peripheral blood CD34⁺ cells from diabetic patients that demonstrated reduced vascular reparative function, we showed that the level of TGF- β , a key factor that modulates stem cell quiescence, was increased in the serum of type 2 diabetic patients and endogenous levels of TGF- β within the EPCs were markedly increased. Transient TGF- β 1 inhibition in CD34⁺ cells improved their reparative ability (cell survival in the absence of added growth factors, SDF-1–induced migration, NO release, and in vivo retinal vascular reparative ability). We concluded that transient inhibition of TGF- β 1 may represent a promising therapeutic strategy for restoring the reparative capacity of dysfunctional diabetic CD34⁺ cells prior to their use in autologous cell therapy [87••].

Other strategies to correct EPC dysfunction to optimize cell therapy include modulation of key antioxidant enzymes. Marrotte et al. [88] report cell therapy using diabetic EPCs after ex vivo manganese superoxide dismutase (MnSOD) gene transfer accelerates their ability to heal wounds in a mouse model of type 2 diabetes.

Angiotensin II regulates blood pressure and contributes to endothelial dysfunction and the progression of atherosclerosis. Bone marrow–derived EPCs in peripheral blood contribute to postnatal vessel repair and neovascularization. Impaired EPC function in patients with hypertension and diabetes inhibits the endogenous repair of vascular lesions and leads to the progression of atherosclerosis. The number of EPCs in peripheral blood is inversely correlated with mortality and the occurrence of cardiovascular events. Angiotensin II–mediated signaling is implicated in oxidative stress, inflammation, and insulin resistance, factors that cause EPC dysfunction. Blockade of the angiotensin II type 1 receptor may therefore present a new therapeutic target for enhancing EPC function [89]. Our group has shown that activation of the protective arm of the renin-angiotensin-aldosterone system (RAAS) in human CD34⁺ EPCs corrects their endogenous dysfunction in diabetes and also restores health to the resident vasculature. The protective axis of the RAAS involves ACE2, which produces angiotensin-(1-7) [Ang-(1-7)], which activates the Mas receptor to stimulate NO production and attenuate ROS [90, 91]. Thus, Ang-(1-7) is a vasodilator peptide with antihypertensive, antihypertrophic, antifibrotic, and antithrombotic functions [92]. We show that diabetic EPCs express low levels of ACE2 but respond robustly to Ang-(1-7), suggesting that Mas receptor function remains intact [93].

Chang et al. [94] showed that adiponectin prevents EPC senescence by inhibiting the ROS/p38 MAPK/p16 (INK4A) signaling cascade and that the protective effects of adiponectin against diabetes vascular complications are attributed in part to its ability to counteract hyperglycemia-mediated decrease in the number of circulating EPCs [94].

Why Limit Cell Therapy to Diabetic Retinopathy? Would Other Ischemic Retinopathies Respond Better than Diabetic Retinopathy?

Although diabetic retinopathy remains the most common ocular ischemic disorder, we have presented evidence that the diabetic milieu impacts significantly on normal EPC function. At the same time, to treat PDR, a treatment must inhibit new retinal blood vessel formation, including blocking EPC recruitment and engraftment (Fig. 1). This, combined with the progressive and long-term nature of the pathology, means that considerably more research is needed to fully understand the nature of the diabetes-related defect in EPCs and how such dysfunction can be reversed. It is important to highlight the fact that diabetic EPCs, whose functionality has been restored in vitro, would still have to be delivered into diabetic patients

and, therefore, inhospitable host diabetic tissues pose another considerable hurdle to overcome.

Retinopathy of prematurity (ROP) is another important ischemic retinopathy, and represents a significant cause of severe visual impairment in childhood. Premature retinas exposed to high ambient oxygen have abnormal regulation of retinal blood flow leading to increased oxygenation with high free radical generation that induces retinal microvascular degeneration, and resulting ischemia triggers aberrant neovascularization [95]. It has been proposed that vascular stem cell therapy is an attractive alternative therapy for ROP [95].

Less complicated ischemic retinopathies such as branch retinal vein occlusion (BRVO) may represent a more appropriate test bed for such vascular stem cell-based therapy. BRVO is relatively common, affecting up to 2% of patients ages 50 to 70 years and despite treatment this disorder carries a significant visual morbidity [96]. Patients typically present with retinal edema, hemorrhage, and congested vessels in the territory of the obstruction. The evolution of the BRVO varies according to the site and order of the occluded vein and efficiency of the collateral circulation to the nearby normal retina. Many BRVOs implicate the macular circulation and cause microvascular insufficiency, edema, and disruption of the central neurons and photoreceptors. With an aging population, BRVO will become increasingly common [96] and more effective treatments are needed [97].

Ideally, active intervention early in the occlusive process will prevent neuroretinal damage particularly at the macula and preserve serviceable vision. Clearly the introduction of EPCs into such a milieu would require pharmacologic manipulation with appropriate anti-inflammatory conditioning of the recipient retina; however, at least in the introduction phase, the presence of significant retinal edema may be beneficial because the expansion of the extracellular space of the retina could overcome the cell-crowding factor in the adult retina and permit the ingress of new intraretinal vasculature. Studies in the kitten ROP model show significant intraretinal vascular proliferation in regions of intraretinal edema [98]. BRVO is an important clinical condition and may represent an easier disease to treat with cell therapy because the area of ischemia is segmented and thus easily defined and any reperfusion of retina can be assessed by fluorescein angiography.

Conclusions

Attempting translational studies in diabetic retinopathy before understanding the full identity and characteristics of EPC phenotype is likely fraught with difficulties. Which cell type is appropriate is still a difficult question to be answered. Although a study by Tendra et al. [99] found no difference in the efficacy of unselected BMCs and CD34⁺CXCR4⁺ cells; preclinical studies and ongoing clinical trials support the use of CD34⁺ cells compared with the total mononuclear leukocytes or unselected BMCs. However, a higher dose of CD34⁺ cells was shown to be less efficacious than lower doses [100]. Autologous cell therapy in diabetic patients requires the ex vivo modification of EPCs for a better reparative outcome regardless of the cell type chosen. We have emphasized the role of NO in maintaining normal function of EPCs/BMCs and ways to maintain balanced NO and ROS levels. All BMC populations may have a key role in the repair process, and it is likely that combinations of progenitor cells will be needed, as will the simultaneous optimization of the diabetic environment into which these cells will be placed. Such a complex approach will likely be needed to optimally treat diabetic patients with retinopathy. Furthermore, stem cell therapy is a promising field for discovering future applications for nondiabetic ocular ischemic disease.

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Fig. 1.

Diabetic dysfunction in the BM mobilization of stem/progenitor cells and paracrine regulation of ischemic vascular repair. In normal conditions, factors released by ischemic/injured tissue cause mobilization of BM cells. In diabetes there is reduced mobilization of BM cells into circulation. Cell therapy in diabetic retinopathy would ideally restore perfusion to areas of the retina that have undergone vasodegeneration associated with NPDR and would prevent the development of advanced disease, PDR. BM—bone marrow; CACs—circulating angiogenic cells; eEPCs—early endothelial progenitor cells; eNOS—endothelial nitric oxide synthase; EPCs—endothelial progenitor cells; EPO—erythropoietin; HSCs—hematopoietic stem cells; IL—interleukin; iNOS—inducible nitric oxide synthase; MCP-1—monocyte chemoattractant protein-1; MnSOD—manganese superoxide dismutase; NO—nitric oxide; NPDR—nonproliferative diabetic retinopathy; OECs—out growth endothelial cells; PDR—proliferative diabetic retinopathy; PPAR-δ—peroxisome proliferator-activated receptor-δ; RAAS—renin-angiotensin-aldosterone system; ROS—reactive oxygen species; SCF—stem cell factor; SDF-1—stromal cell-derived factor-1; TGF-β—transforming growth factor-β; TNF-α—tumor necrosis factor-α; VEGF—vascular endothelial growth factor